The following oligonucleotides were used in the construction of plasmids.

Table 2: Oligonucleotides Utilized For *LovE* Variant Cloning
MO664 (5'GGCCATGGAGGCCGCTAGCTCGAGTCGACGGCCTAGGTGGCCAGCT3') (SEQ ID NO:1)
MO665 (5'GGCCACCTAGGCCGTCGACTCGAGCTAGCGGCCTCCATGGCCGTAC3') (SEQ ID NO:2)
MO666 (5'GGCGGCCGCTCTAGAACTAGTCTCGAGGGTACC3') (SEQ ID NO:3)
MO667 (5'GGTACCCTCGAGACTAGTTCTAGAGCGGCCGCC3') (SEQ ID NO:4)
MO1794 (5' CACAGCGGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:5)
MO1793 (5'CACCACTAGTACGCGGGCTGATTCGAC3') (SEQ ID NO:6)
MO1785 (5'CACCACTAGTTATACATTATATAAAGTAATGTG3') (SEQ ID NO:7)
MO1786 (5'CACAGGATCCGTCATCTTTGCCTTCGTTTATC3') (SEQ ID NO:8)
MO195 (5'CGCGGATCCTATTGAACAAGATGGATTGCAC3') (SEQ ID NO:9)
MO196 (5'CCGGAATTCAGAAGAACTCGTCAAGAAG3') (SEQ ID NO:10)
MO841 (5' ACAAAAAAGCAGGCTCCACAATGGCTGCAGATCAAGGTAT3') (SEQ ID NO:11)
MO842 (5' ACAAGAAAGCTGGGTTCATGGAGGAATATTGTTGA3') (SEQ ID NO:12)
MO2278 (5' GGGGATCCAATCGAGGTCCACGACCAGT3') (SEQ ID NO:13)
MO343 (5' GGGGACAAGTTTGTACAAAAAAGCAGGCT3') (SEQ ID NO:14)
MO2273 (5' GGGGATCCGCCAATGGTCCCGTTCAAAC3') (SEQ ID NO:15)
MO2274 (5' ACAAGAAAGCTGGGTTCACAGAATGTTTAGCTCAA3') (SEQ ID NO:16)
MO344 (5' GGGGACCACTTTGTACAAGAAAGCTGGGT3') (SEQ ID NO:17)
MO2624 (5'GCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGG3') (SEQ ID NO:18)
MO2654 (5'CGTCGCGCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:19)
MO2680 (5'GGACCTTTGCAGCATAAATTACTATACTTCT3') (SEQ ID NO:20)
MO2686 (5'GGCGCGTCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:21)
MO2681 (5'TAAAACTCTTGTTTTCTTCTTTTCTCTAAAT3') (SEQ ID NO:22)
MO2700 (5'CAGTGAGCGCGCGTAATACGACTCACTATAGGGCGA3') (SEQ ID NO:23)
MO2701 (5' ATACTTCTATAGACACACAAACACAAATACACACAC3') (SEQ ID NO:24)
MO107 (5'CGCGGATCCCGTCGTTTTACAAC3') (SEQ ID NO:25)
MO197 (5'CCCAAGCTTATTATTTTTGACACCAGACCAA3') (SEQ ID NO:26)
MO1293 (5'GGAAGATCTAGCATCGTGGCCAATTTCTTCTAGTTT3') (SEQ ID NO:27)
MO1294 (5'ATAAGAATGCGGCCGCTCAACCTTCCCATTGGGGCGTTTGC3') (SEQ ID NO:28)
MO1787 (5'CACAGGATCCAGCATTATTAATTTAGTGTGTGTATTT3') (SEQ ID NO:29)
MO1788 (5'CACCACTAGTCTCGAGCAGATCCGCCAG3') (SEQ ID NO:30)
MO1793 (5'CACCACTAGTACGCGGGCTGATTCGAC3') (SEQ ID NO:31)
MO1794 (5'CACAGCGGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:32)
MO511 (5'GGCCATCGATACAAGTTTGTACAAAAAAGCTGAAC3') (SEQ ID NO:33)
MO540 (5'GGCGCCCTATTACACCACTTTGTACAAGAAAGC3') (SEQ ID NO:34)
MO1985 (5'CACACGTCTCCGGCCTCAACCTTCCCATTGGGGCG3') (SEQ ID

NO:35)
MO1986 (5'CACACAGATCTCGTGGCCAATTTCTTCTAGTTTGA3') (SEQ ID NO:36)
MO1992 (5'CACACGGATCCACAATGTTACGTCCTGTAGAAACCCC3') (SEQ ID NO:37)
MO1993 (5'CACAGCGGCCGCTTCATTGTTTGCCTCCCTGCTG3') (SEQ ID NO:38)
MO316 (5'GCGGCCGCGGCGCCCGGCCCATGTCAACAAGAAT3') (SEQ ID NO:39)
MO318 (5'CCGCGGCCGAGTGGAGATGTGGAGT3') (SEQ ID NO:40)

Plasmid MB2254 contains the *lovF*p-*HIS3*p-*neo* reporter gene flanked by *URA3* sequence. First primers MO664 (SEQ ID NO:1) and MO665 (SEQ ID NO:2) were annealed and inserted into the *Kpn*I-*Sac*I sites of plasmid pBluescript II KS (Stratagene,). The resulting vector, MB1038, contains a *Sal*I site in the polylinker. Next, the *Spe*I-*Xho*I fragment from pJL164 (Brachmann *et al. Yeast* **14**:115-132 (1998)) containing a deletion of the *URA3* gene with additional flanking sequences was inserted into the *Nhe*I-*Sal*I sites of MB1038 to create MB1053. Primers MO666 (SEQ ID NO:3) and MO667 (SEQ ID NO:4) that contain multiple restriction sites (*Not*I, *Xba*I, *Spe*I, *Xho*I and *Kpn*I) were then annealed together and ligated into the *Sma*I site of MB1053 to create MB1054. Next, the following four fragments were combined in MB1054 to obtain plasmid MB2254. The *lovF* promoter from *A. terreus* genomic DNA was PCR amplified with MO1794 (SEQ ID NO:5) and MO1793 (SEQ ID NO:6) and inserted into MB1054 on a *Not*I-*Spe*I fragment. The *HIS3* basal promoter from pRS403 (Sikorski and Hieter, *Genetics* **122**:19-27 (1989)) was PCR amplified with primers MO1785 (SEQ ID NO:7) and MO1786 (SEQ ID NO:8) and inserted into MB1054 on a *Spe*I-*Bam*HI fragment. Finally, the *neo* gene (PCR amplified with MO195 (*Bam*HI) (SEQ ID NO:) and MO196 (*Eco*RI) (SEQ ID NO:10) from plasmid pYX11 (Xiao and Weaver, *Nucl. Acids Res.* **25**:2985-2991 (1997)) and *CYC1* terminator sequences (*Xho*I-*Kpn*I fragment from pRS426-*GAL*-S (Mumberg, *et al.*, *Nucl. Acids. Res.* **22**:5767-5768 (1994)) were first combined in pRS416 (Sikorski and Hieter,

Genetics **122**:19-27 (1989)) and then cut out with *Bam*HI-*Kpn*I and inserted into MB1054 to create MB2254.

The *lovF*p-*HIS3*p-*neo* reporter in MY2124 can confer resistance to the drug geneticin (G418). It was empirically determined that MY2124 (untransformed or transformed with parental plasmids MB2478 (*CYC1-lovE/CEN*) or MB2848 (*CYC1-lovE/At274/CEN*) was unable to grow on YPD media supplemented with 100 µg /ml G418. Plasmid MB2478 contains the *CYC1* promoter operationally linked to the entire *A. terreus lovE* open reading frame. The *CYC1* promoter is a relatively weak promoter and thus the *lovE* ORF in MB2478 was expressed at low levels. MB2478 was the parental vector plasmid for creating full length *lovE* variants. Plasmid MB2848 contains the *CYC1* promoter operationally linked to a chimeric open reading frame consisting of the *A. terreus lovE* DNA binding domain fused to the carboxy-terminal portion of the *At274* gene (U.S. Serial No. 60/257,431, filed December 22, 2000).

MB2848 was used to create *lovE* variants in which the DNA binding domain was not mutated. Both MB2478 and MB2848 contain yeast CEN and autonomously replicating sequences and both are maintained at 1-2 copies per cell. In contrast to strains transformed with MB2478 or MB2848, strains transformed with plasmid MB1644 (*TEF1-lovE*/2 micron) were able to grow on G418-supplemented YPD media. The *lovE* gene of MB1644 is under control of the constitutively strong *S. cerevisiae TEF1* promoter. MB1644 contains a 2-micron origin for high-copy replication in yeast. An objective of these studies was to identify *lovE* variants which when expressed at low levels could confer G418 resistance similar to the highly expressed wild-type *lovE* molecule of MB1644. *S. cerevisiae* expression vectors used in these studies were constructed as follows.

MB968 is a low copy *S. cerevisiae URA3* based expression vector. MB968 was created by inserting the *Eco*RV fragment (containing the destination cassette) from gateway pEZC7201 (Invitrogen™, Carlsbad, CA) into